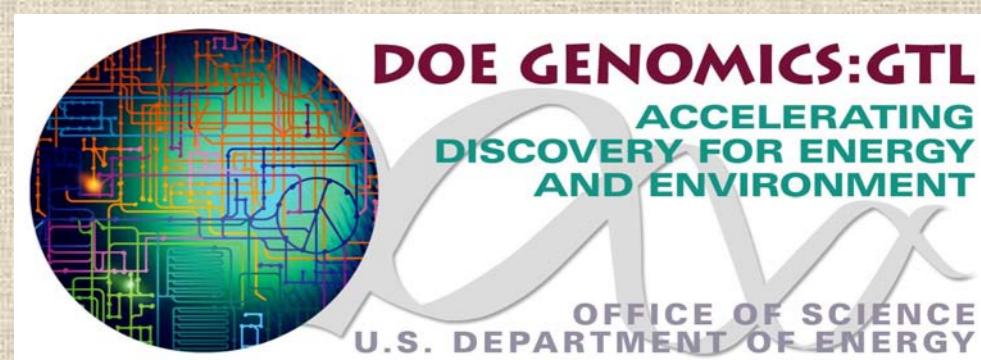


Integrative studies of *Desulfovibrio vulgaris* and a hydrogenotrophic methanogen *Methanococcus maripaludis* growing in syntrophic association: What's being bartered?



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Abstract

As part of the DOE GTL initiative evaluating the use of microbial stress responses to monitor the status of environmental systems, we are developing laboratory reactors that simulate environmental conditions unattainable in pure culture. Although sulfate-reducing prokaryotes (SRP) characteristically respire sulfate, their distribution does not appear to be restricted by sulfate availability. In the absence of sulfate, some SRPs can grow syntrophically with hydrogenotrophic methanogens. Interspecies hydrogen transfer is known as a driving process for such interactions, but transfer of formate or other compounds might also occur. Initial studies have characterized the growth of *Desulfovibrio vulgaris* Hildenborough (DvH) syntrophically coupled to a hydrogenotrophic methanogen (*Methanococcus maripaludis*) on a lactate medium without sulfate. Transcriptional analyses of *D. vulgaris*, grown as co- and mono-cultures in chemostats, were performed with a "Meta-Genome Oligonucleotide (70mers) Array" covering all ORFs of the *D. vulgaris* and *M. maripaludis* genomes (3574 and 1766 oligonucleotides respectively). This array was designed and manufactured at ORNL. A preliminary metabolic model was constructed using flux balance analysis to provide a framework for analyzing the experimental data.

Goals

- i) to study metabolite and gas evolution during syntrophic growth of DvH and methanogen *M. maripaludis*
- ii) use a DNA microarray to reveal gene expression profile in co-culture
- iii) use stoichiometric modeling to test our predictions and experimental data

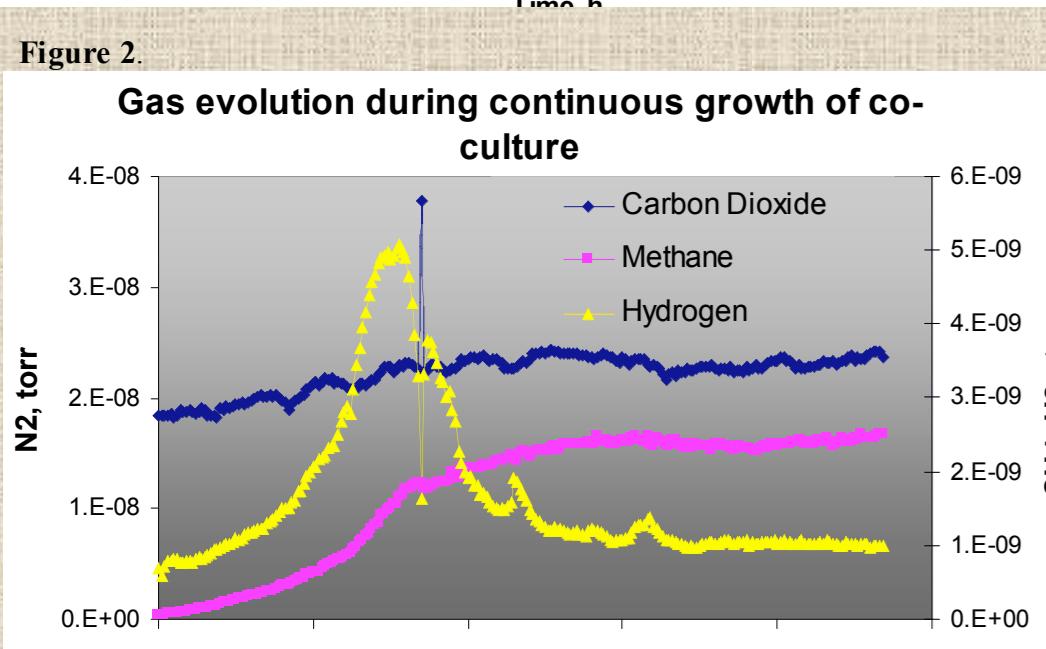
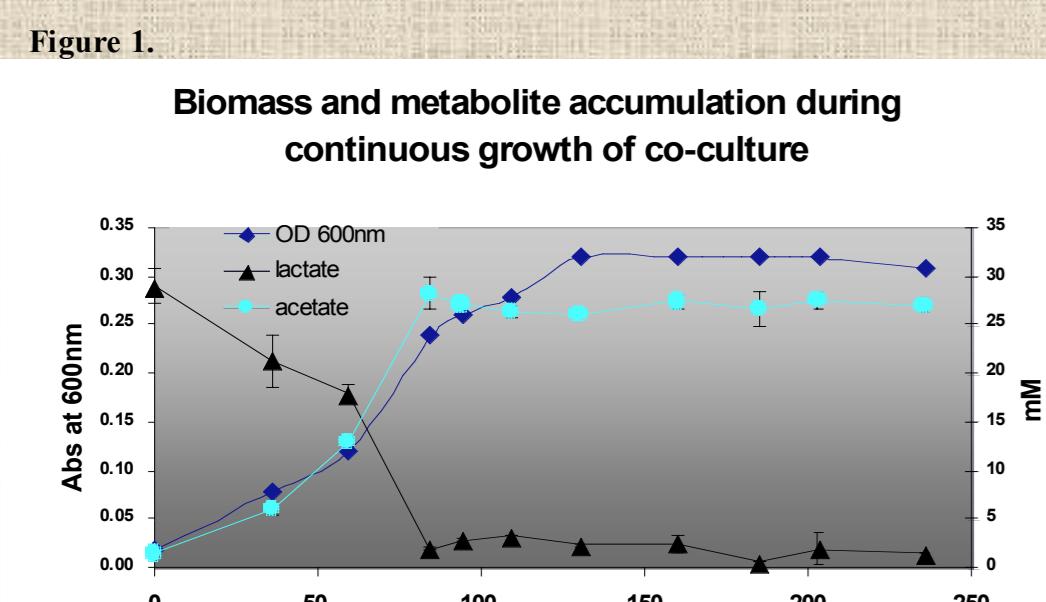
Growth and physiology of syntrophic co-culture

DvH cells for physiological and comparative gene expression studies were grown in chemostats under the following two conditions with a 10% CO₂/90% N₂ headspace.

1. DvH alone in sulfate limiting conditions:
2 Lactate + SO₄²⁻ → 2 Acetate + H₂S + 2HCO₃⁻

2. DvH in co-culture with *M. maripaludis* in the absence of sulfate.
2 Lactate + H₂O → 2 Acetate + CH₄ + H⁺ + HCO₃⁻

Optical densities, metabolite concentrations and gas evolution during a typical continuous process under growth condition 2 are presented in Figures 1 and 2.



Stoichiometric modeling

To complement and direct experimental studies on the physiology of *Desulfovibrio* growing either alone or in co-culture, a metabolic model was constructed using flux balance analysis (FBA). The *Desulfovibrio* model consists of 86 reactions and 73 internal metabolites, while that of the methanogen contains 84 reactions and 72 metabolites. For the chemostat experiment, lactate uptake and acetate production rates were calculated based on the feed lactate concentration (27 mM), volumetric flowrate (0.086 L/hr), and the measured concentrations in the effluent. Rates were averaged over the time period of 130–235 hours, since the data indicates the chemostat maintained steady-state during this time. Two different scenarios were tested: electron transfer between the two species using H₂ and formate, and electron transfer using only H₂. The rate of H₂ transfer was predicted to be significantly higher in the absence of formate transfer. The biomass yield for both organisms was predicted to be the same in each scenario; therefore, H₂ and formate are interchangeable from an energetic point of view. However, *M. maripaludis* can not grow using formate only. Some H₂ transfer is necessary, and the problem becomes infeasible if this reaction is eliminated.

Figure 3a. Simulation of co-culture growth with H₂ and formate exchange.

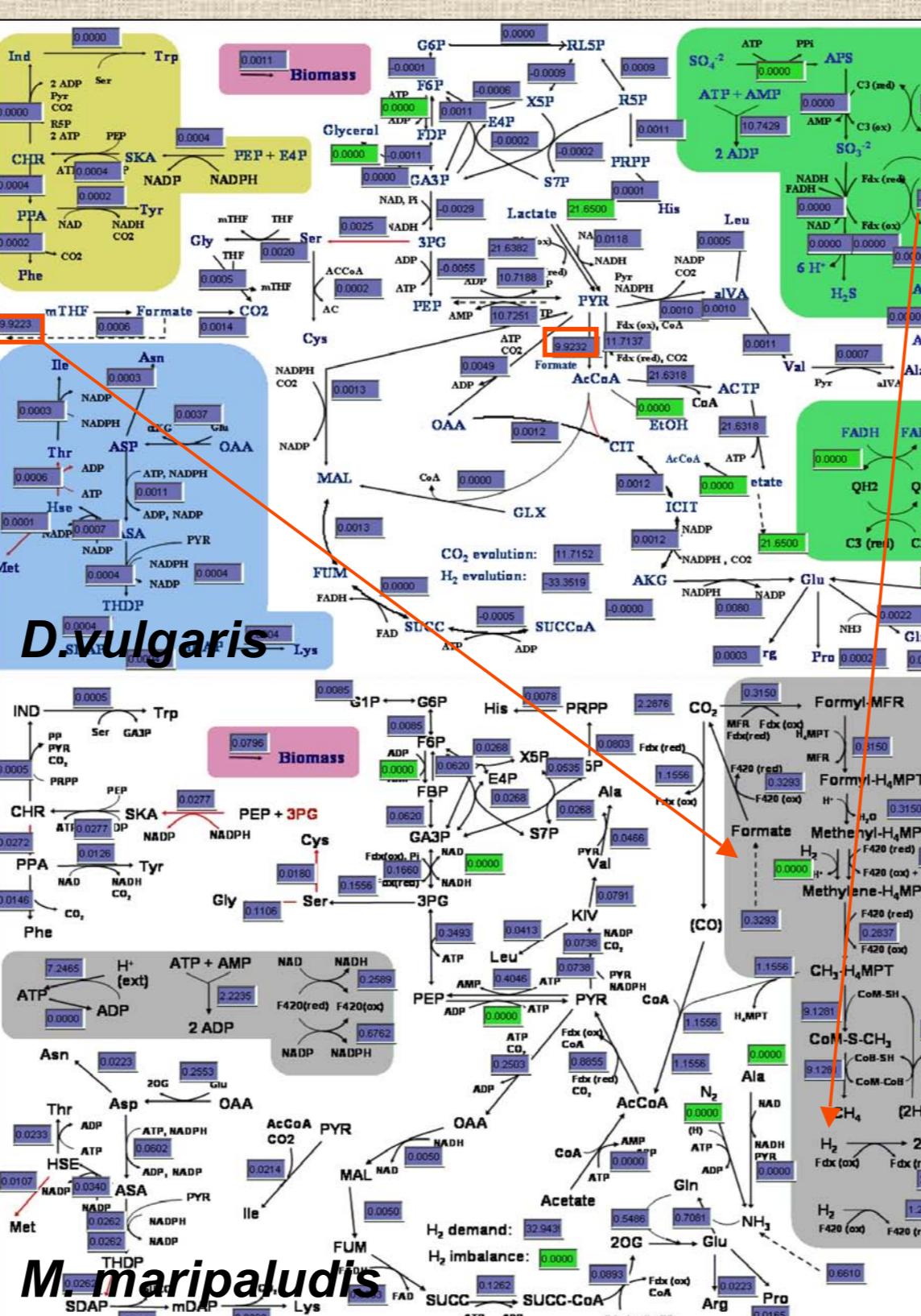
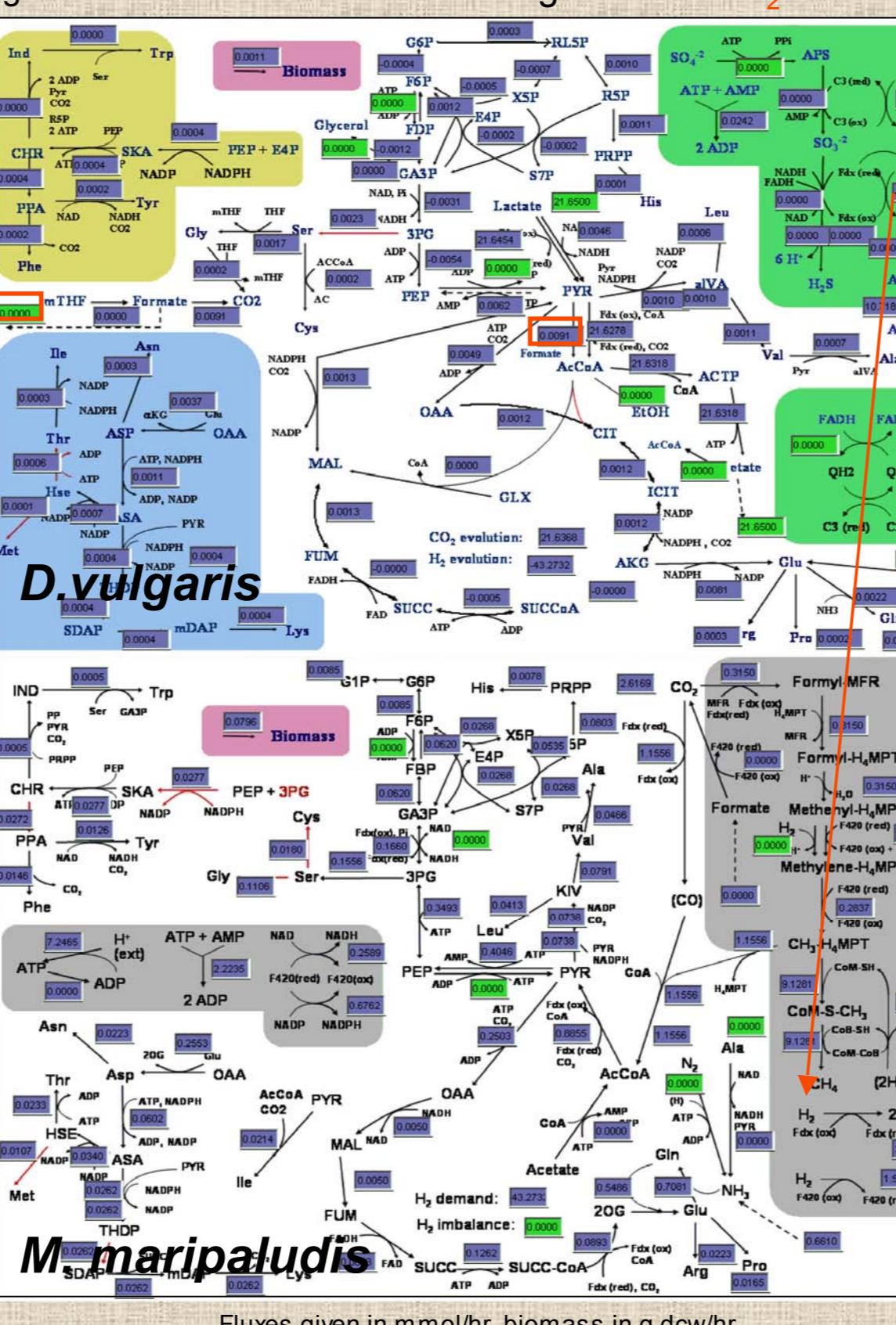


Figure 3b. Simulation of co-culture growth with H₂ exchange.



Gene expression analysis

For comparative studies, DvH mono-cultures were grown in a chemostat under sulfate limitation (30 mM lactate and 10 mM sulfate) with a 10% CO₂/90% N₂ headspace, or in syntrophic co-culture without sulfate, with a dilution rate 0.042 h⁻¹, and collected at steady-state. *M. maripaludis* was grown in chemostat in McC medium amended with amino acids and acetate with hydrogen and carbon dioxide in the head space with a dilution rate of 0.13 h⁻¹. Total RNA from mono- or co-cultures labeled with Cy3 dye and genomic DNA of both organisms labeled with Cy2 dye by random priming were used for hybridization to the oligonucleotide array.

An initial experiment (three technical reps) has provided preliminary data for understanding the physiology of syntrophic growth. Because we currently have a single biological replicate, we only consider genes with significant changes in expression level and genes in operons demonstrating synchronized change in expression level.

DvH cells grown in co-culture showed the most significant increase in expression of several genes encoding a response regulator (DVU0145, 6-fold) and membrane and lipoproteins that are most likely organized in an operon (DVU0147, DVU0149, DVU0150, DVU0153, 5 to 12-fold). Cells of methanogens and sulfate reducers tend to aggregate in the co-culture, and products of these genes may encode surface proteins responsible for the cell adhesion.

List of *M. maripaludis* genes upregulated in co-culture

MMP0824	4.5	conserved F420-reducing hydrogenase subunit beta
MMP0823	4.3	conserved F420-reducing hydrogenase subunit delta
MMP0817	4.2	conserved F420-reducing hydrogenase subunit beta
MMP0975	4.1	possible multi-domain ABC transporter substrate-binding protein
MMP0909	3.9	formylmethanofuran dehydrogenase subunit A
MMP0830	3.6	conserved F420-reducing hydrogenase subunit alpha
MMP0819	3.5	conserved F420-reducing hydrogenase subunit delta
MMP0835	3.5	conserved hypothetical protein
MMP0899	3.5	conserved F420-reducing hydrogenase subunit gamma
MMP0818	3.4	formate transporter
MMP151	3.3	conserved B12-binding/Cobalamin-dependent methionine synthase
MMP0878	3.3	formate dehydrogenase-related protein
MMP1537	3.1	Cytochrome
MMP1538	3.0	proton transporter component
MMP1552	2.9	hypothetical protein
MMP0152	2.8	hypothetical Fd reductase subunit B2
MMP0106	2.8	conserved hypothetical protein
MMP0346	2.6	2-hydroxybutyrate-CoA dehydrogenase related protein
MMP0154	2.5	heterodisulfide reductase subunit C2
MMP0372	2.5	F420-dependent methenyltetrahydromethanopterin dehydrogenase
MMP0931	2.4	conserved hypothetical protein
MMP0658	2.4	conserved F420-dependent NS,N10-MHPP reductase

Conclusions

We expected genes for sulfate reduction and some periplasmic dehydrogenases to be down-regulated in DvH during growth in syntrophic co-cultures. Conversely, hydrogen producing (cytoplasmic) hydrogenases were expected to be over-expressed.

Consistent with Expectations

- One DvH gene cluster coding for a putative heterodisulfide reductase and a hydrogenase was upregulated in co-culture.

Deviation from Expectations

- Genes for one cytoplasmic hydrogenase (Coo) showed a decrease in expression.
- DvH genes encoding proteins for sulfate reduction are expressed during syntrophic growth.

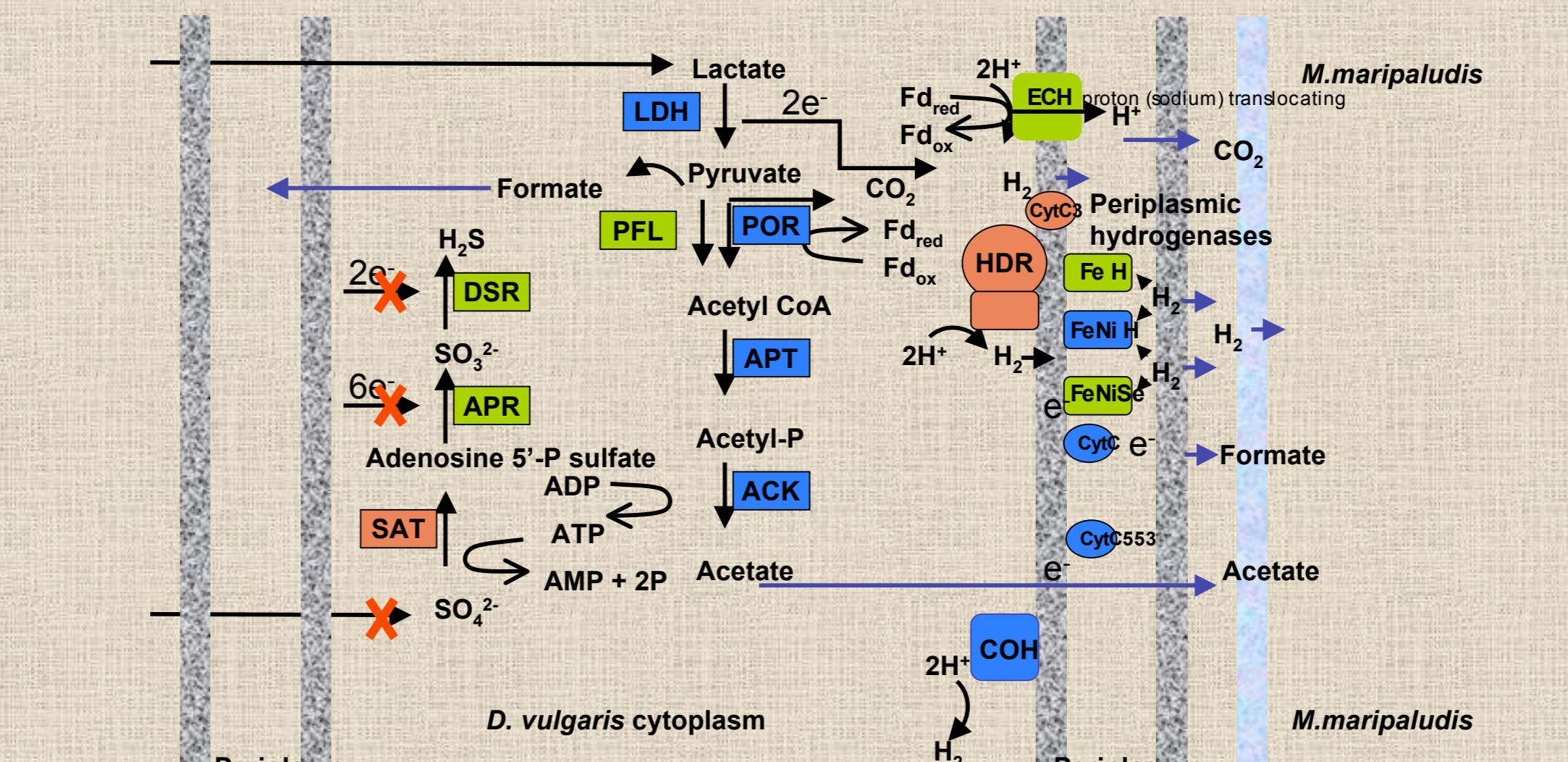
Additional Observations

- Several operons containing genes involved in energy generation were found to be upregulated in *M. maripaludis* growing in co-culture.
- Gene for a formate transporter was upregulated in *M. maripaludis*

Metabolic Modeling

- Use of stoichiometric models in combination with experimental data can help characterize the metabolism and energetics of both organisms in syntrophic co-culture.
- Simulations suggest that both hydrogen and formate can act as interspecies electron carriers in co-culture.

Visualization of chemostat co-culture microarray data overlaid on *D. vulgaris* metabolic pathway model



Colored shapes represent proteins. Red indicates upregulation, blue indicates downregulation, and green indicates no change in gene expression in co-culture. Red X indicates absence of a flux through the sulfate reduction pathway (DvH is not reducing sulfate in the co-culture environment).

DSR – dissimilatory sulfite reductase; APR – APS reductase; SAT – sulfate adenyllyl transferase; LDH – lactate dehydrogenase; POR – Pyruvate ferredoxin oxidoreductase; APT – acetyl P transferase; ACK – acetate kinase; HDR – heterodisulfide reductase (hydrogenase); ECH – energy conserving hydrogenase; Fe H – Fe containing hydrogenase; FeNi H – Fe Ni containing hydrogenase; FeNiSe – Fe Ni and selenocysteine containing hydrogenase; COH – CO induced hydrogenase; Cyt – cytochrome; PFL – pyruvate formate lyase

Formate may play a role in syntrophic co-culture.

Formate was detected neither in batch nor in chemostat co-cultures. A formate dehydrogenase deficient *M. maripaludis* mutant was able to grow in co-culture with DvH. While interspecies formate transfer may occur in syntrophic interactions, as suggested by the observed upregulation of a *M. maripaludis* gene coding for a formate transporter (see table on the right), the importance of such transfer remains unclear.

Acknowledgments

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